

Docket No. MWH-0029US

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Stephen B. Liggett  
Application No.: 09/856,803  
Filed: May 25, 2001 (35 U.S.C. § 371 of PCT/US99/27963, filed November 24, 1999, which claims benefit of U.S. Appl. No. 60/109,886, filed November 25, 1998)  
Confirmation No.: 3706  
Group No.: 1634  
Examiner: Myers, C.  
For: POLYMORPHISMS IN THE 5' LEADER CISTRON OF THE  $\beta_2$ -ADRENERGIC RECEPTOR

Commissioner for Patents  
Washington, D.C. 20231

Certificate of Facsimile Transmission  
I hereby certify under 37 C.F.R. § 1.8 that this correspondence is being transmitted by facsimile to the United States Patent and Trademark Office, Commissioner for Patents, TC 1600, at (703) 872-9306, on July 7<sup>th</sup>, 2003.

  
Matthew M. Catlett

DECLARATION OF STEPHEN B. LIGGETT, M.D., UNDER 37 C.F.R. § 1.131

This Declaration Of Stephen B. Liggett, M.D., Under 37 C.F.R. § 1.131 is being submitted as part of Applicant's Response To Office Action Under 37 C.F.R. § 1.11 regarding the office action dated January 7, 2003 that was received in the captioned application.

Being warned that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001, and that such willful false statements and the like may jeopardize the validity of the instant application or patent resulting therefrom, I hereby declare that:

1) I am the original, sole, and first inventor of the subject matter that is claimed in pending claims 1-8 and 11 of the captioned application, namely (a) a method for genotyping the

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$\beta_2$ -adrenergic receptor ( $\beta_2$ AR) gene of an individual comprising determining the identity of the nucleotide pair at the 5' leader cistron (5'LC) polymorphic site (PS), which, as is demonstrated throughout the specification of the captioned application, is located 47 bases upstream of the  $\beta_2$ AR coding region, which begins at nucleotide position 1588 of SEQ ID NO:1 (thus, the 5'LC PS is located at nucleotide position 1541 of SEQ ID NO:1) in the two copies of the  $\beta_2$ AR gene present in the individual; and (b) a method for genotyping the  $\beta_2$ AR gene of an individual comprising determining the identity of the nucleotide pair at the 5'LC PS and at one or more additional PSs in the  $\beta_2$ AR gene in the two copies of the  $\beta_2$ AR gene present in the individual.

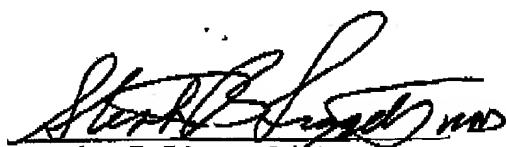
2) Further to an effort, dating back to as early as January of 1996 (see attached copies of PCR protocols), to discover polymorphisms in the region upstream of the  $\beta_2$ AR gene, I directed the performance of an experiment designed to elucidate the existence, if any, of such polymorphisms. Utilizing PCR techniques to analyze genomic DNA in this region from human volunteers, I discovered, in the "sense" strand, the existence of a thymine residue 47 bases upstream of the  $\beta_2$ AR coding region, as well as the existence of an adenine residue 47 bases upstream of the  $\beta_2$ AR coding region in the "antisense" strand. Copies of chromatograms generated by the automated sequencer used to sequence the PCR products demonstrating this discovery are attached (chromatogram #096-1369 demonstrates a thymine residue in the "sense" strand at the nucleotide position that is located 47 bases upstream of the  $\beta_2$ AR coding region; chromatogram #096-1364 demonstrates an adenine residue in the "antisense" strand at the nucleotide position that is located 47 bases upstream of the  $\beta_2$ AR coding region; chromatogram #096-1367 demonstrates a thymine residue in the "sense" strand at the nucleotide position that is located 47 bases upstream of the  $\beta_2$ AR coding region; and chromatogram #096-1362 demonstrates an adenine residue in the "antisense" strand at the nucleotide position that is located 47 bases upstream of the  $\beta_2$ AR coding region). Although all previous reports indicated that the only known residue at the nucleotide position located 47 bases upstream of the  $\beta_2$ AR

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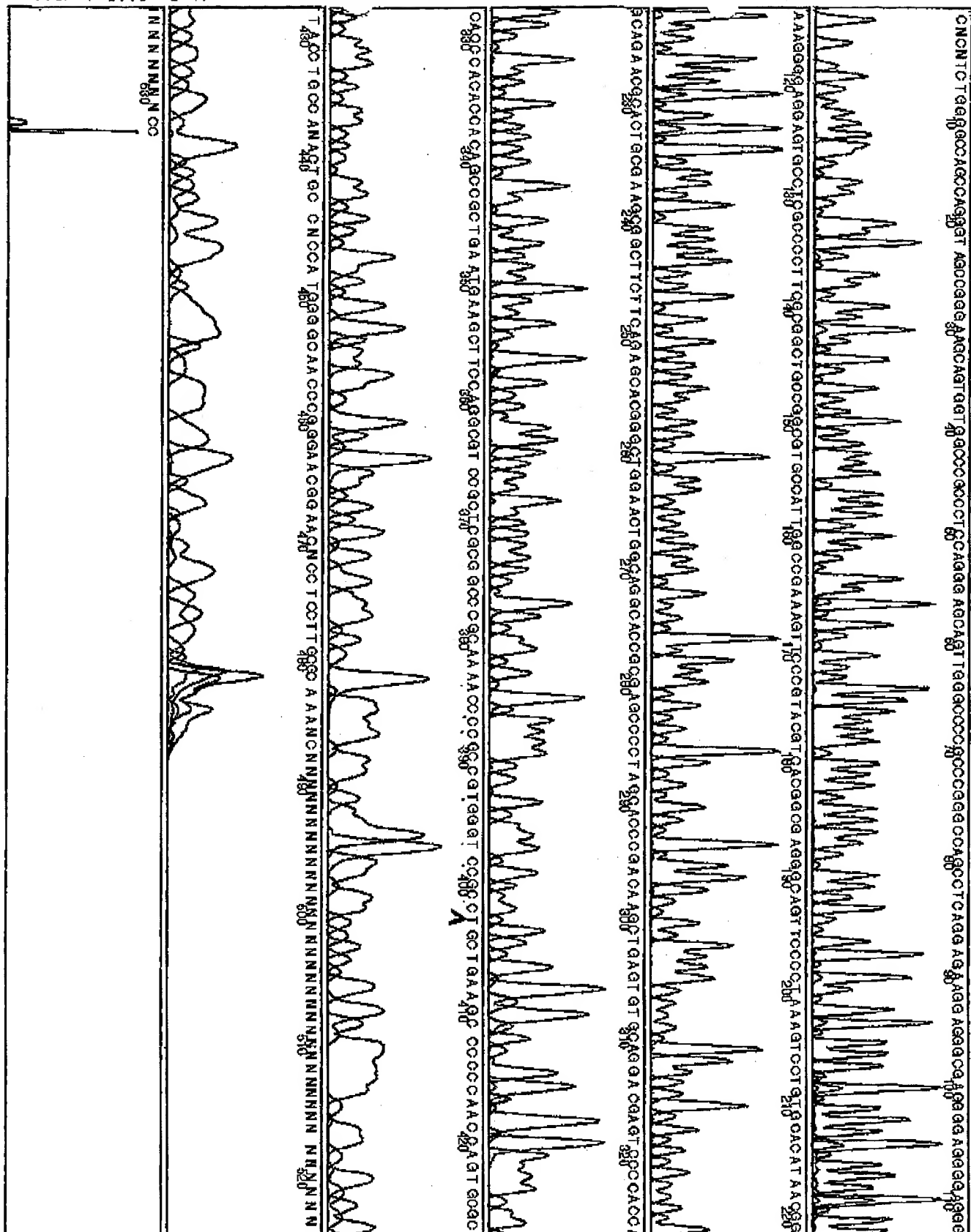
PATENT

coding region, in the "sense" strand, was a cytosine (and thus, in the "antisense" strand, a guanine), to confirm that I had indeed discovered a polymorphism at this position, I subsequently directed the performance of a similar experiment with the wild-type sequence, and discovered, in the "sense" strand, a cytosine, and in the "antisense" strand, a guanine. Copies of chromatograms generated by the automated sequencer used to sequence the PCR products demonstrating this discovery are attached (chromatogram #096-2859 demonstrates a cytosine residue in the "sense" strand at the nucleotide position that is located 47 bases upstream of the  $\beta_2$ AR coding region; and chromatogram #096-2860 demonstrates a guanine residue in the "antisense" strand at the nucleotide position that is located 47 bases upstream of the  $\beta_2$ AR coding region). My discovery of this polymorphism, and my subsequent confirmation of this discovery, occurred prior to the effective date of any of the following references: Timmermann *et al.*, *Kidney Intl.* 53:1455-60 (June 1998), Timmerman *et al.*, *J. Molecular Med.* 76:B30, Abst. P-109 (May 1998), Timmermann *et al.*, *Human Mutation* 11(4):343-4 (March 1998). With respect to the copies of the chromatograms, the nucleotide position that is 47 bases upstream of the  $\beta_2$ AR coding region is that denoted with a "^" symbol.

3) All statements made herein of my knowledge are true, and all statements made herein on information and belief are believed by me to be true.



Stephen B. Liggett, M.D.  
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JUL 7. 2003 2:49PM

GENAISSANCE PHARM.

NO. 953

P. 20



Model 373A  
Version 2.0.1S

Hb/H 18  
Dye Terminator/Any Primer  
Lane 16

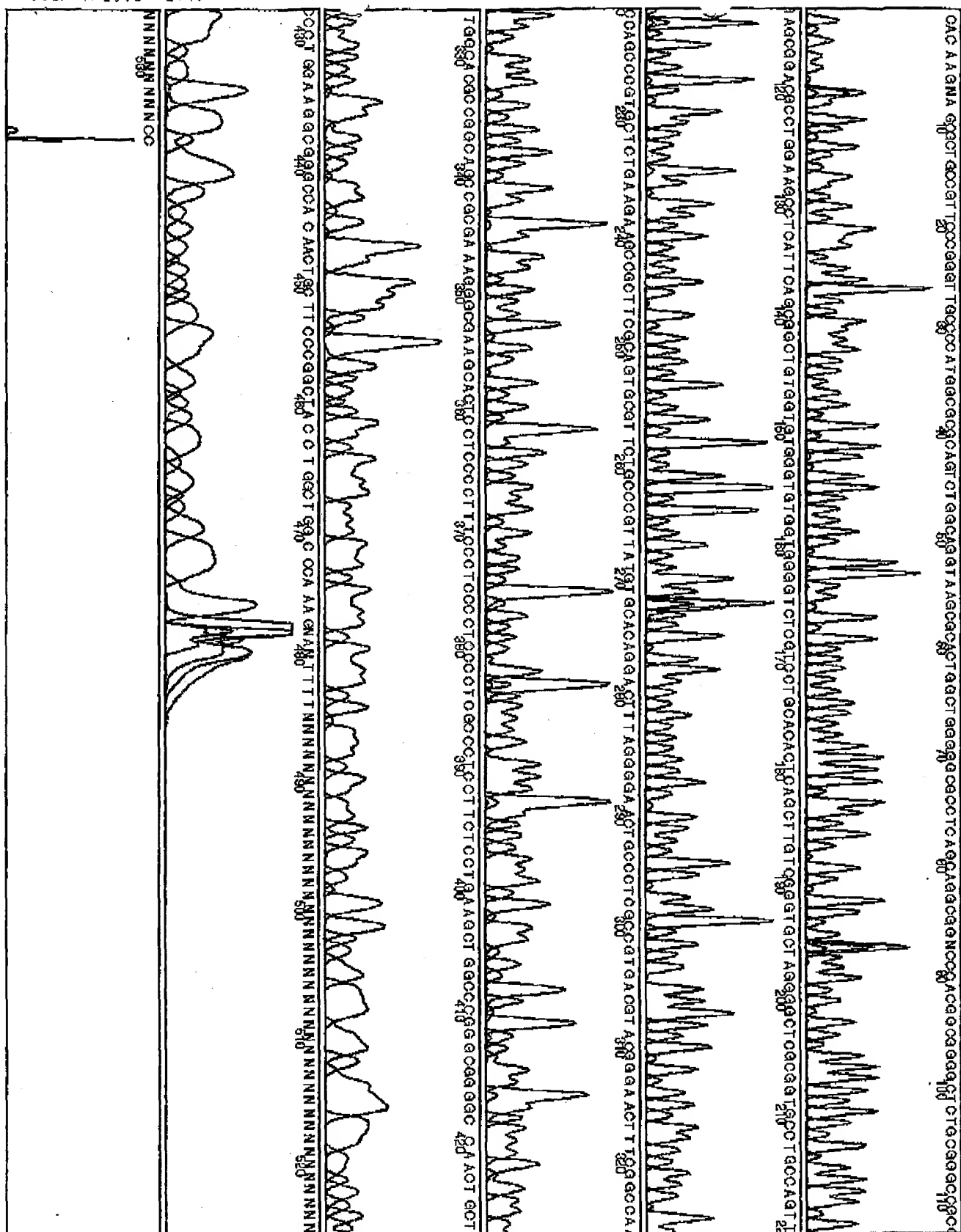
Signal: G:1186 A:1244 T:584 C:580

INST FILE 308296  
096-1364  
MCGRAW-A-2R-1

Tue, Mar 5, 1996 8:51 PM  
X: 0 to 6840 Y: 0 to 1600  
Spacing: 10.38

Page 11

A2-R







Model: 373A

Version 2.0.15

B635 U8  
 Dye Terminator (Any Primer)  
 Lane 8  
 Signal: G:406 A:399 T:97 C

INST FILE 808286  
086-2859  
MOGRA W/A9F1

Thu, May 9, 1996 4:57 PM  
X: 0 to 6472 Y: 0 to 1600  
Spacing: 10.26

Page 1

Signal: G:406 A:399 T:97 C:121

**MOGRAPH 44/48/1**

[illegible]

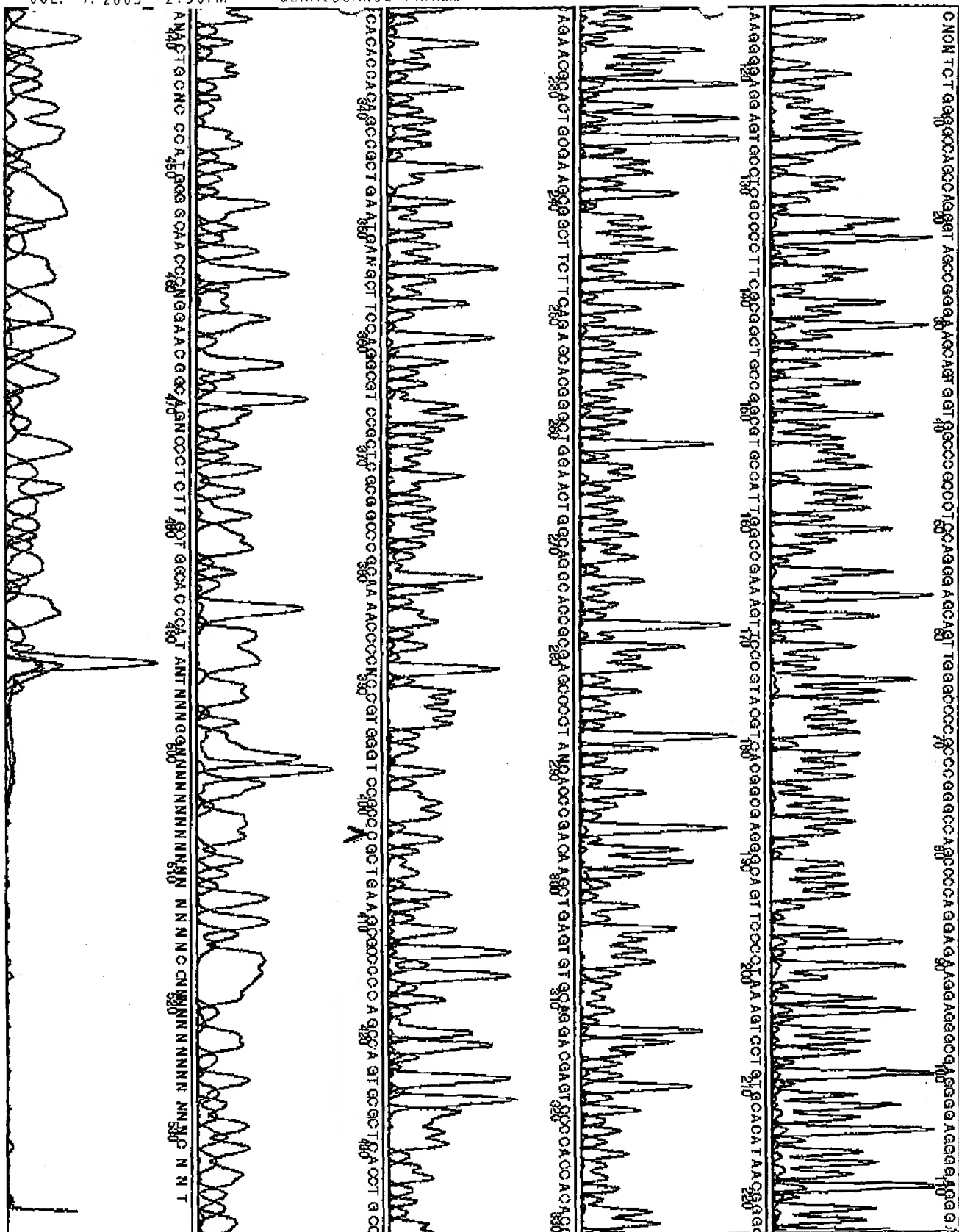
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NO. 953

GENAISSANCE PHARM.

2:50 PM

JUL 7 2003







Model 373A

Version 2.0.1S

Beck DU  
Dye Terminator (Any Primer)  
Lane 9

Signal: G:315 A:191 T:85 C:85

INST FILE 808296  
086-2860  
MOCRAV#A9R1

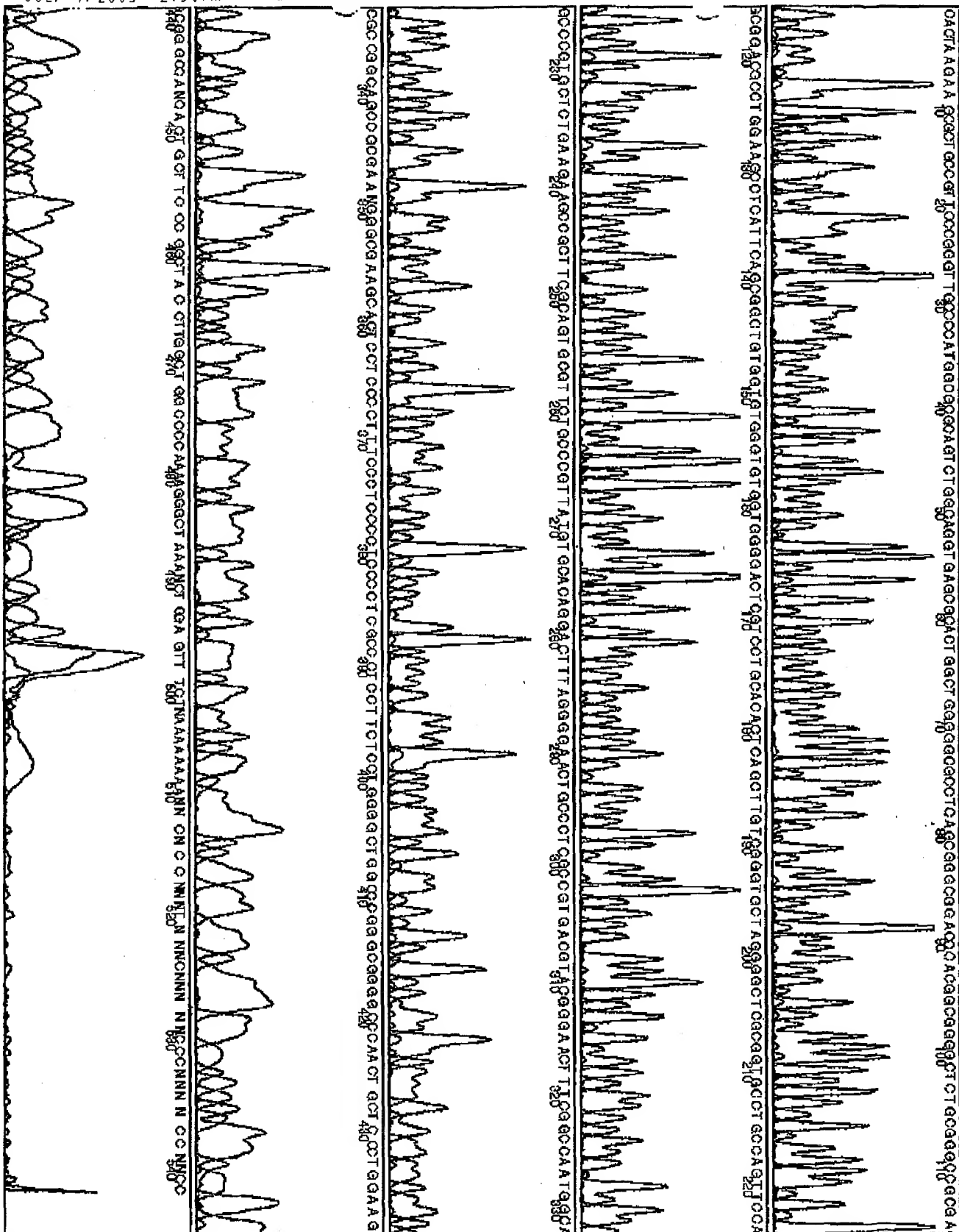
Thu, May 9, 1996 4:57 PM  
X: 010 6452 Y: 010 1600  
Spacing: 10.31

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GENAISSANCE PHARM.

JUL 7 2003 2:50PM



PCR (B2AR)

1/26/96

\* Made new set of primers for B2AR 5' flanking region. ~~Both~~  
Product should include the CRE & short ORF. Primers  
were chosen = MacVector

Blood samples are being obtained from pts in the UC cell line  
clinic by Melanie Meyers.

DNA samples prepared by Dig Donnelly using Autogen + 10  
Diana kits. Samples are numbered as received (A1, A2, etc.)

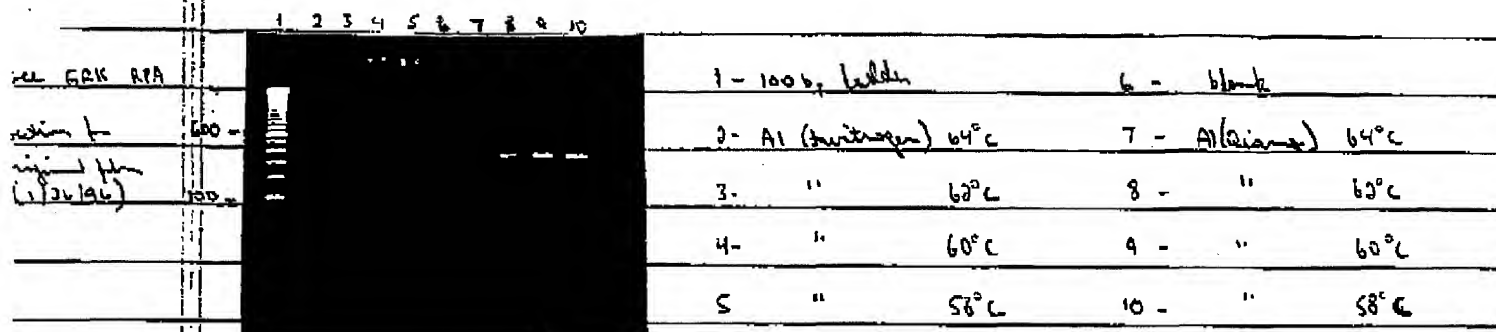
- Set up PCR rxn using new primers. Made up 96ul master mix.
  - 10ul buffer II
  - 6ul 25mM MgCl<sub>2</sub>
  - 0.8ul 25mM dNTP
  - 0.5ul 100uM forward primer (B2AR-F1)
  - 0.5ul 100uM reverse primer (B2AR-R1)
  - 77.7ul dH<sub>2</sub>O
  - 0.5ul amplitag
- digest 24ul of master mix into 2 PCR tubes
- add 1ul template DNA
- overlay = 1 drop mineral oil
- perform PCR in thermocycler = 1st gradient made on
  - 94°C x 2 min
  - 94°C x 30 sec / 64°C, 62°C, 60°C, or 58°C x 30 sec / 72°C x 30 sec => 35 cycles
  - 72°C x 7 min
- run 10ul of PCR rxn on TBE minigel

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GENAISSANCE PHARM.

NO. 953 P. 26

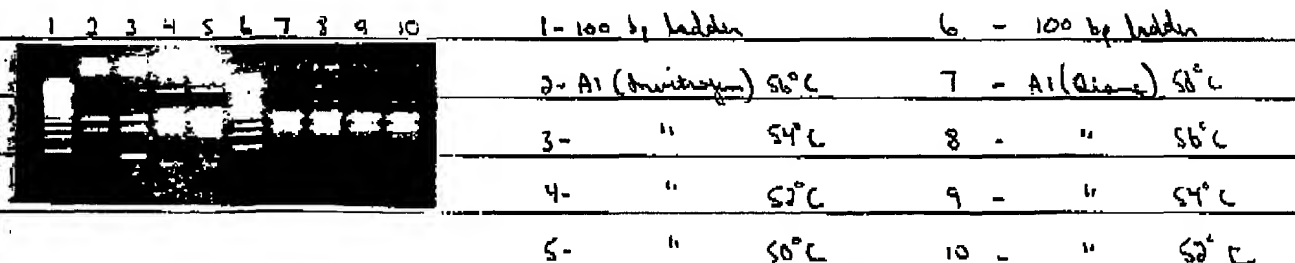
1/26/96 (cont)



No product seen = DNA isolated by Amiragen kit. Nice band seen = DNA from Qiana kit but expected size should be 538 bp. The band present appears to be < 400 bp.

Repeat PCR with following modifications:

- add 73.7 ul dH<sub>2</sub>O
- aliquot 23 ul of master mix into PCR tubes
- add 2 ul template DNA
- change annealing temp for Amiragen template to 56°, 54°, 52°, 50°C; change annealing temp for Qiana template to 58°, 56°, 54°, 52°C.



Now have bands in all samples. However, apparent band present in both samples sets appears to be < 500 bp, still smaller than expected.

B2AR PCR (5-tube)

3/1/96

Set up master mix for four (4) 25- $\mu$ l PCR rxns:

2 $\mu$ l	deplete DNA (DWM)
1.5 $\mu$ l	forward primer
1.5 $\mu$ l	reverse primer
20 $\mu$ l	SX buffer (buffer N from Stratagene PCR optimization kit)
10 $\mu$ l	dUTP <sub>s</sub> (2.5mM) (from optimization kit)
65 $\mu$ l	dH <sub>2</sub> O
0.8 $\mu$ l	Tag
100 $\mu$ l	total

- aliquot 25  $\mu$ l of master mix into 4 PCR rxn tubes- overlay  $\approx$  1 drop of mineral oil

- PCR cycle	98°C	2 min	} 30 cycles
	98°C	30 sec	
	56° 54° 53° 50°	30 sec	
	72°C	30 sec	
	72°C	7 min	

- remove 10  $\mu$ l aliquot & run on minigel

#1 - 100 bp ladder

#2 - 56°C

#3 - 54°C

#4 - ~~56~~ 53°C

#5 - 50°C

